In Vivo ESR Spin trapping of Acute Aflatoxin B1-Induced Metabolism in Rats

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Oxidative damage has been postulated to play a major role in the mechanisms associated with aflatoxin B1-induced cytotoxicity and carcinogenecity in mammalian species. An elegant approach of detecting free radicals *in vivo*, developed by Mason and co-investigators, involves the direct examination of trapped radicals from biological fluids, such as bile, urine and plasma. The aim of this study was to detect and identify free radical intermediates from the hepatic metabolism of aflatoxin-B1 *in vivo*. Rat bile ducts were cannulated and rats were treated simultaneously with aflatoxin-B1 (3 mg/kg i.p.) and the spin trapping agent 4-POBN (?(4-pyridyl-1-oxide)-*N*-*tert*-butyl nitrone) (1 g/kg i.p.), and bile was collected over a period of 2 hours. ESR spectroscopy was used to detect a carbon-centered radical adduct of 4-POBN in rat bile, urine and liver following acute exposure to AFB1. Detection of 4-POBN radical adducts in bile, urine and liver samples provides strong evidence for the generation of aflatoxin-B1-metabolism induced free radicals during acute exposure to AFB1. The effect of metabolic inhibitors, such as deferoxamine mesylate (DFO), an iron chelator, and SKF 525A, a cytochrome P-450 inhibitor, on *in vivo* aflatoxin-induced free radical formation was also studied. Both DFO, an iron chelator, and SKF525A, a cytochrome P-450 inhibitor, significantly decreased radical formation from AFB1 metabolism, which implies a mixed function oxidase dependence as well as the involvement of iron (probably via the Fenton reaction) in AFB1-induced radical generation in mammals.